

chain variable region gene to the exon of the human gamma chain constant region gene using the polymerase chain reaction. Subsequently, the 31.1 chimeric gene was cloned into a retroviral expression vector pLgptCXII and transfected into the packaging cell line PA317. The transfected cells (PA317H) were cultivated with another packaging cell line PA317L, which contained an irrelevant mouse/human chimeric light chain gene in retroviral expression vector pLneoCXII and SP2/0-Ag14 cells. The transduced SP2/0-Ag14 cells yielded a complete chimeric antibody, Chi #1 which reacted with horseradish peroxidase-conjugated IgG of goat anti-human IgG Fc in ELISA analyses, which indicated that the constant region of Chi #1 was human. Cytofluorometry analysis indicated that Chi #1 stained human colorectal carcinoma cell lines HT-29 and LS174T but not a human lung carcinoma cell line A-427. Antibody-dependent cell-mediated cytotoxicity (ADCC) assay indicated that Chi #1 lysed LS174T cells. These results show that Chi #1 retained the antigen-binding specificity of the parental 31.1 mouse monoclonal antibody, suggesting the usefulness of this chimeric antibody in ascertaining prognosis of colon carcinoma.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

What is claimed is:

1. A monoclonal antibody specific for a purified human colon carcinoma-associated protein antigen, wherein said antigen has the following characteristics:

- (a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;
- (b) said antigen is not detectable on normal colon cancer free human tissues;
- (c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;
- (d) said antigen is specifically immunogenic in humans; and
- (e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity.

2. An antibody according to claim 1 which is mouse monoclonal antibody 33.28 (ATCC HB-12315) or an antibody which binds specifically to a colon carcinoma-associated epitope that specifically binds to monoclonal antibody 33.28.

3. An antibody according to claim 2 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of about 61.1 kilodaltons.

4. An antibody according to claim 1 which is mouse monoclonal antibody 31.1 (ATCC HB-12314) or an antibody which binds specifically to a colon carcinoma-associated epitope that specifically binds to monoclonal antibody 31.1.

5. An antibody according to claim 4 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of about 72 kilodaltons.

6. An antibody according to claim 2 wherein said colon carcinoma-associated antigen is a glycoprotein, the protein component having a molecular weight of 61.1 kilodaltons.

7. An antibody according to claim 1 immobilized on a solid phase.

8. An antibody according to claim 1 which is detectably labelled.

9. An antibody according to claim 8 wherein said detectable label is a radiolabel.

10. An antibody according to claim 1 conjugated to a cytotoxic radionuclide.

11. An antibody according to claim 1 conjugated to a cytotoxic drug.

12. An antibody according to claim 1 conjugated to a cytotoxic protein.

13. A composition comprising an antibody according to claim 10 in combination with a pharmaceutically acceptable carrier.

14. A composition comprising an antibody according to claim 11 in combination with a pharmaceutically acceptable carrier.

15. A composition comprising an antibody according to claim 12 in combination with a pharmaceutically acceptable carrier.

16. A monoclonal antibody against the monoclonal antibody of claim 1.

17. A monoclonal antibody against the monoclonal antibody of claim 2.

18. A monoclonal antibody against the monoclonal antibody of claim 3.

19. A monoclonal antibody against the monoclonal antibody of claim 4.

20. A monoclonal antibody against the monoclonal antibody of claim 5.

21. A monoclonal antibody against the monoclonal antibody of claim 6.

22. An immunoassay for detecting a colon carcinoma-associated antigen which binds to mouse monoclonal antibody 33.28 (ATCC HB-12315) in a sample comprising:

- (a) contacting said sample with an effective binding amount of the antibody according to claim 1; and
- (b) detecting said antigen by detecting the binding of the antibody to the purified colon carcinoma associated protein antigen.

23. An immunoassay for detecting a colon carcinoma-associated antigen which binds to mouse monoclonal antibody 31.1 (ATCC HB-12314) in a sample comprising:

- (a) contacting said sample with an effective binding amount of the antibody according to claim 1; and
- (b) detecting said antigen by detecting the binding of the antibody to the purified colon carcinoma associated protein antigen.

24. A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having a colon cancer;
- (b) contacting the specimen with monoclonal antibody 33.28 (ATCC HB-12315);
- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex by the stain.

25. A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having colon-carcinoma;
- (b) contacting the specimen with mouse monoclonal antibody 31.1 (ATCC HB-12314);
- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex.

26. A method according to claim 24 wherein the stain is an avidin-biotin immunoperoxidase stain.

27. A method according to claim 25 wherein the stain is an avidin-biotin immunoperoxidase stain.

28. A kit for the immunohistochemical detection of colon carcinoma comprising:

- (a) mouse monoclonal antibody 31.1 (ATCC HB-12314);
- (b) reagents for immunoperoxidase and secondary antibody;
- (c) immunoperoxidase; and
- (d) colorizing reagents.

29. A kit for the immunohistochemical detection of colon carcinoma comprising:

- (a) mouse monoclonal antibody 33.28 (ATCC HB-12315);
- (b) reagents for immunoperoxidase and secondary antibody;
- (c) immunoperoxidase; and
- (d) colorizing reagents.

30. A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, wherein the antigen has the following characteristics:

- (a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;
- (b) said antigen is not detectable on normal colon cancer free human tissues;
- (c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;
- (d) said antigen is specifically immunogenic in humans; and
- (e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,

said kit comprising a first container adapted to contain an antibody to said antigen or an active component thereof, and a second container adapted to contain a second antibody to said antigen or an active component thereof, said second antibody being labeled with a reporter molecule capable of giving a detectable signal.

31. A kit according to claim 30 wherein the reporter molecule is a radioisotope, an enzyme, a fluorescent molecule, a chemiluminescent molecule or a bioluminescent molecule.

32. A kit according to claim 30 wherein the reporter molecule is an enzyme.

33. A kit according to claim 30 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.

34. A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, wherein the antigen has the following characteristics:

- (a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;
- (b) said antigen is not detectable on normal colon cancer free human tissues;

- (c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;
- (d) said antigen is specifically immunogenic in humans; and

(e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,

said kit comprising a first container adapted to contain monoclonal antibody 31.1 (ATCC HB-12314) to said antigen and a second container adapted to contain a second antibody to said antigen or an active component thereof, said second antibody being labeled with a reporter molecule capable of giving a detectable signal.

35. A kit according to claim 34 wherein the reporter molecule is a radioisotope, an enzyme, a fluorescent molecule, a chemiluminescent molecule or a bioluminescent molecule.

36. A kit according to claim 32 wherein the reporter molecule is an enzyme.

37. A kit according to claim 33 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.

38. A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, wherein the antigen has the following characteristics:

- (a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;
- (b) said antigen is not detectable on normal colon cancer free human tissues;
- (c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;
- (d) said antigen is specifically immunogenic in humans; and
- (e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,

said kit comprising a first container adapted to contain monoclonal antibody 33.28 (ATCC HB-12315) to said antigen and a second container adapted to contain a second antibody to said antigen or an active component thereof, said second antibody being labeled with a reporter molecule capable of giving a detectable signal.

39. A kit according to claim 38 wherein the reporter molecule is a radioisotope, an enzyme, a fluorescent molecule, a chemiluminescent molecule or a bioluminescent molecule.

40. A kit according to claim 38 wherein the reporter molecule is an enzyme.

41. A kit according to claim 38 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.

42. The monoclonal antibody of claim 1 which is a chimeric antibody.

43. The chimeric antibody according to claim 42 which is a chimeric mouse/human antibody Chi #1 (ATCC CRL-12316).

44. The chimeric antibody according to claim 42 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of 72 kilodalton.

45. A composition comprising the chimeric antibody according to claim 42 in combination with a pharmaceutically acceptable carrier.

46. A monoclonal antibody against the chimeric antibody of claim 42.

47. An immunoassay for detecting a colon carcinoma-associated antigen which binds to the mouse/human chi-

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meric antibody Chi #1 (ATCC CRL-12316) of claim 42 in a sample comprising:

(a) contacting said sample with the antibody according to claim 42; and

(b) detecting said antigen by detecting the binding of said antibody to the purified colon carcinoma associated protein antigen. 5

48. A method for diagnosing colon cancer in humans comprising:

(a) removing a histological specimen from a patient suspected of having a colon carcinoma; 10

(b) contacting the specimen with a chimeric antibody which binds to an antigen according to claim 1;

(c) staining the specimen with an immunohistochemical stain; and 15

(d) detecting the presence of the antigen-antibody complex by the stain.

49. A method for diagnosing colon cancer in humans comprising:

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(a) removing a histological specimen from a patient suspected of having a colon carcinoma;

(b) contacting the specimen with mouse/human chimeric antibody which binds to an antigen which binds to mouse/human chimeric antibody Chi #1 (ATCC CRL-12316);

(c) staining the specimen with an immunohistochemical stain; and

(d) detecting the presence of the antigen-antibody complex by the stain.

50. A kit for the immunohistochemical detection of colon carcinoma comprising:

(a) mouse/human chimeric antibody Chi #1 (ATCC CRL-12316);

(b) reagents for immunoperoxidase and secondary antibody;

immunoperoxidase; and

(d) colorizing reagents.

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